showed avoidance of fields/meadows in spring ($\chi^2 = 28.63$, df = 4, $p < 0.001$) and used habitats in proportion to their availability during summer ($\chi^2 = 1.75$, df = 4, $p = 0.78$).

The average annual home range size of 1662 ha in this study was larger than ranges reported for radiocollared male wildcats in previous studies (Corbett 1979; Stahl et al. 1988). However, MCP seasonal range sizes in this study were smaller than seasonal MCP range sizes recorded for male wildcats in Switzerland (Liberek 1999), indicating that home range sizes in male European wildcats vary under different ecological conditions. Use of home ranges is believed to be exclusive in areas where lagomorphs dominate the diet as opposed to exclusiveness only for animals of the same sex in areas where rodents dominate the diet (Stahl et al. 1988). As the energetic requirements of wildcats increase with the number of prey items required to fulfil their energy demand (Hemmer 1993), the observed differences in range sizes may be explained by differences in availability and abundance of prey.

First order selection (Johnson 1980) of areas inhabited by European wildcats have highlighted the importance of large forested areas with clearings interspersed to increase the amount of edge (e. g. Vogt 1985; Hemmer 1993). First order selection would be strongly influenced by persecution by people and resulting extirpation from large areas of suitable habitat.

Third order selection using radio-telemetry has been studied in east central France (Stahl 1986). Results of this study are consistent with Stahl (1986) with seasonal total ranges including 40 to 66% of forested areas showing the importance of cover to wildcats. Because 65 to 74% of the locations encompass more than 1 habitat category, the importance of habitat boundaries, especially forest edge was indicated. The animals studied showed individual and seasonal variation in habitat selection. The flexibility of wildcat habitat use during this study indicates the ability of wildcats to live in forested landscapes altered by humans and suggests that habitat may not be preventing wildcat recovery from low population numbers.

Acknowledgements

The presented project was part of a study conducted at the Institute of Biogeography at the University of the Saarland, Germany. I thank Dr. P. Müller, Institute of Biogeography, University of the Saarland for financial support and added telemetry data and H. Carius for telemetry field work. I am thankful to Dr. Bruce McLellan, Dr. Urs Breitenmoser, and Roger Ramcharita for comments on earlier drafts of this manuscript. Dr. Anne Olson and Dr. Dan Weary provided helpful comments on statistical analyses.

References

Corbett, L. K. (1979): Feeding ecology and social organization of wildcats (*Felis silvestris*) and domestic cats (*Felis catus*) in Scotland. Diss. thesis, University of Aberdeen, U. K.

Fischer, H. (1989): Rheinland-Pfalz und Saarland – Eine geographische Landeskunde. Darmstadt: Wiss. Buchgesellschaft.

Hatlapa, H.; Wiesener, H. (1982): Die Praxis der Wildtierimmobilisation. Hamburg: Paul Parey.

Hemmer, H. (1993): *Felis silvestris* Schreber, 1777 – Wildkatze. In: Handbuch der Säugetiere Europas. Ed. By J. Niethammer and F. Krapp. Wiesbaden: Akad. Verlagsges. Vol. 5/II, 1076–1118.

Johnson, D. H. (1980): The comparison of usage and availability measurements for evaluating resource preference. Ecology **61**, 65–71.

Kenward, R. E. (1995): Ranges V: An analysis system for biological location data. User manual, Wareham, Dorst, U. K.: Institute of Terrestrial Ecology.

Liberek, M. (1999): Eco-Ethologie du chat sauvage *Felis s. silvestris* Schreber, 1777 dans le Jura Vaudois (Suisse); Influence de la couverture neigeuse. Diss. thesis, Université de Neuchâtel, Switzerland.

Mohr, C. O. (1947): Table of equivalent populations of North American small mammals. Am. Midl. Nat. **37**, 223–249.

Neu, C. W.; Byers, C. R.; Peek, J. M. (1974): A technique for analysis of utilization-availability data. J. Wildl. Manage. **38**, 541–545.

Piechocki, R. (1990): Die Wildkatze. Wittenberg: A. Ziemsen Verlag.

Sládek, J. (1973): Jahreszeitliche und jahresbedingte Veränderung der Nahrung der Wildkatze (*Felis silvestris* Schreber, 1777) in den Westkarpaten. Zool. Listy **22**, 127–144.

Stahl, P. (1986): Le Chat forestier d'Europe (*Felis silvestris* Schreber, 1777): exploitations des ressources et organisation spatiale. Diss. thesis, Université Nancy I, France.

Stahl, P.; Artois, M.; Aubert, M. F. A. (1988): Organisation spatiale et déplacements des chat forestiers adultes (*Felis silvestris* Schreber, 1777) en Lorraine. Rev. Ecol. **43**, 113–132.

Vogt, D. (1985): Aktuelle Verbreitung und Lebensstätten der Wildkatze (*Felis silvestris silvestris* Schreber, 1777) in den linksrheinischen Landesteilen von Rheinland-Pfalz und Beiträge zu ihrer Biologie. Beiträge Landespflege Rheinland-Pfalz **10**, 130–165.

White, G. C.; Garrott, R. A. (1990): Analysis of wildlife radio-tracking data. New York: Academic Press.

Worton, B. J. (1989): Kernel methods for estimating the utilization distribution in home-range studies. Ecology **70**, 164–168.

Author's address:

Heiko U. Wittmer, Faculty of Agricultural Sciences, University of British Columbia, #270 – 2357 Main Mall, Vancouver, B. C., Canada, V6T 1Z4 (e-mail: wittmer@interchange.ubc.ca)

Mamm. biol. **66** (2001) 371–375
© Urban & Fischer Verlag
http://www.urbanfischer.de/journals/mammbiol

Mammalian Biology
Zeitschrift für Säugetierkunde

Short communication

Genetic distinction of roe deer (*Capreolus pygargus* Pallas) sampled in Korea

By H. S. Koh and E. Randi

Department of Biology, Chungbuk University, Korea and Istituto Nazionale per la Fauna Selvatica, Ozzano dell'Emilia, Bologna, Italia

Receipt of Ms. 17. 01. 2001
Acceptance of Ms. 30. 05. 2001

Key words: *Capreolus pygargus*, mitochondrial DNA, molecular taxonomy

The roe deer (genus *Capreolus*, Artiodactyla, Cervidae) includes two species: the smaller European (*C. capreolus*) and the larger Siberian (*C. pygargus*) roe deer (Groves and Grubb 1987; Sokolov and Gromov 1990; Hewison and Danilkin 2001), which have widespread distributions in central-western Europe, and in Asia and east Europe, respectively. They show a number of diagnostic morphologic, chromosomal and DNA traits (Groves and Grubb 1987; Heptner et al. 1989; Douzery and Randi 1997; Randi et al. 1998 a). Populations of the two species occur in putative contact zones in the Caucasus, where *C. capreolus* is distributed at the southern slopes and *C. pygargus* at the northern slopes of the mountain ranges, and along the rivers Volga and Don (Heptner et al. 1989; Danilkin 1996). However, most probably European and Siberian roe deer populations do not hybridize in nature where they overlap in distribution (Danilkin 1996; Hewison and Danilkin 2001).

Populations of the Siberian roe deer show extensive body size and coat colour variability, and up to six subspecies were recognized (Grubb 1993). However, recently Danilkin (1996) and Hewison and Danilkin (2001) recognized only two subspecies: *C. p. pygargus*, from western and part of eastern Siberia, and *C. p. tianschanicus*, from Tian Shan and eastern Asia, including Korea. Other authors referred to roe deer populations from Korea as a distinct subspecies, e. g., *C. p. bedfordi* (Sokolov and Gromov 1990), or *C. p. ochracea* (Barclay 1935). The genetic basis and taxonomical significance of morphological variation among Siberian roe deer populations are still unclear. Aim of this study is to assess the genetic distinction and clarify the taxonomic status of the *C. pygargus* population from Cheju Island in Korea.

Three specimens of roe deer from Cheju Island in Korea were collected and muscle samples were stored in a deep-freezer. Total cellular DNA was extracted from muscle samples digested for 2 hours at 55 °C in 500 µl of STE buffer (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 25 µl of 10 mg/ml Proteinase K stock solution, and 25 µl of 20% SDS solution. DNA was extracted with equal volumes of phenol-chloroform and chloroform, precipitated with 2 volumes of ethylalcohol, and resuspended in 50 µl of distilled water. The solution was incubated at 37 °C for 30 min with 5 µl of 10 mg/ml RNase stock solution, and DNA was extracted again.

The entire mtDNA CR was PCR-amplified using primers L-Pro and H-Phe (Douzery

1616-5047/01/66/06-371 $ 15.00/0.

and Randi 1997; Randi et al. 1998 b) and the following thermal cycle: 94 °C for 5 min; 94 °C for 1 min, 72 °C for 1 min, 54 °C for 1 min (32 cycles); 72 °C for 5 min. PCR-amplified products were purified using the DNA PrepMateTM kit (Bioneer Co., Cheongwon-gun, Korea), and sequenced using an automated DNA sequencer (Perkin Elmer 377) at the Korea Basic Science Institute (Daejeon).

The new mtDNA CR sequences were aligned with homologous complete (Douzery and Randi 1997) and partial (Randi et al. 1998 b) sequences, using the computer program ClustalX (Thompson et al. 1997). Phylogenetic analyses were performed by maximum parsimony (MP), using paup* 4.0b 2a (Swofford 1998), with unordered character states (uninformative nucleotide positions excluded), 10 replicates of random addition of terminal sequences and TBR branch swapping, and by neighbor-joining procedure (NJ; Saitou and Nei 1987), with Tamura and Nei (1993) DNA distances (TN93), using PAUP*. Majority-rule (50%) consensus trees were constructed when multiple equally parsimonious topologies resulted from MP analyses. Robustness of the phylogenies was assessed by bootstrap percentages (BP; Felsenstein 1985), with 1000 random resamplings. Clades can be considered significantly supported when BP values are ≥ 70%.

PCR products were clean single bands of the expected molecular weight, and the sequencing allowed unambiguous nucleotide identifications. Regional organization of the CR from *Capreolus* was concordant with data from other cervid CRs (Douzery and Randi 1997; Randi et al. 1998 b; Nagata et al. 1998; Cook et al. 1999; Randi et al. 2001), thus suggesting that they represent true mtDNA sequences and not nuclear copies. The CR of *Capreolus* includes a central conserved region (CR-II), a left domain (CR-I, on the tRNAs Thr and Pro side), and a right domain (CR-III, on the tRNA Phe side; not shown).

A complete alignment 926 nucleotide long was obtained (the new sequences are available from the GenBank with accession nos. AJ311188 and AJ311189). The average TN93 genetic distance between the Cheju samples and European or Siberian roe deer was 4.5%, and 2.5%, respectively. Roe deer from Cheju showed two distinct mtDNA CR haplotypes, which diverged at 0.2% of their sequences. Moreover, we have aligned partial mtDNA CR sequences, 679 nucleotide long, obtained from 23 samples of European, Siberian, and Korean roe deer. Equally weighted MP analysis of the CR alignment (with 47 parsimony-informative characters) produced 90 trees (length L = 81; consistency index CI = 0.617; retention index RI = 0.881). The 50% majority-rule consensus tree is equivalent to the NJ phylogenetic tree, which is shown in Fig. 1.

MP and NJ trees concordantly showed that: (1) European and Siberian roe deer sequences split into two divergent evolutionary lineages (BP = 100%); (2) Siberian roe deer sequences split into three significantly different evolutionary lineages (BP = 70%–100%), including roe deer from east Siberia (Amur Region), Korea, and west Siberia (Kurgan Region); and (3) roe deer sequences from Korean individuals joined into a monophyletic dade (supported by BP = 91%), which was nested within the Siberian roe deer clade which is the sister clade of the roe deer sampled in the Kurgan Region (west Siberia).

Thus, mtDNA sequences support a genetic distinction between west Siberian and far eastern roe deer, in accordance with both Sokolov and Gromov (1990) and Danilkin (1996) taxonomical listings. The new mtDNA sequences analysed in this study strongly suggest that roe deer from Cheju island are more closely related to populations sampled from the geographically distant Kurgan Region in west Siberia (*C. p. pygargus*) than to those from the geographically close Amur region in south-east Siberia (Fig. 1). The mtDNA sequences from Cheju Island form a significantly distinct clade within *C. pygargus*, thus indicating that roe deer from Korea belong to distinct populations, and supporting Sokolov and Gromov's (1990) view that roe deer from

the far east including Korea, should be kept distinct from the other Siberian roe deer populations. The Korean peninsula is a part of eastern Asia within the Palaearctic region, and it is bound on the north by northeastern China and far eastern Russia. Cheju Island, the largest of the southern Korea islands, originated by a series of volcanic ac-

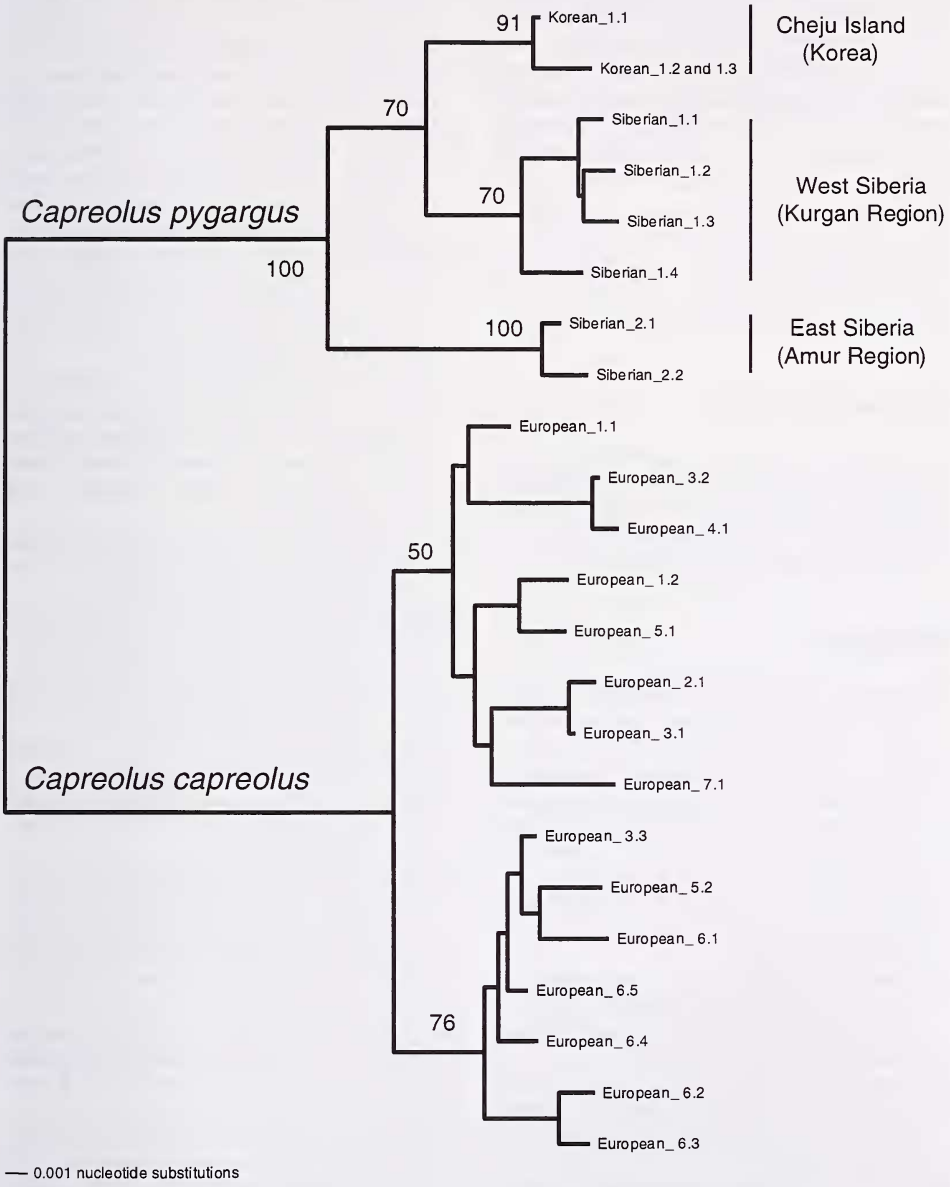

Fig. 1. Neighbor-joining tree (rooted using homologous *C. elaphus* mtDNA CR sequences as outgroups; not shown) clustering mtDNA CR haplotypes from *Capreolus*, computed using TAMURA and NEI (1993) DNA distances. At internodes are reported the bootstrap values ≥ 50%. For the identification of *C. pygargus* (Siberian) and *C. capreolus* (European) haplotypes, see: RANDI et al. 1998 b.